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OUTPUT OF POTASSIUM FROM
THE HEART.

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THE EFFECT OF VAGUS INHIBITION ON THE OUTPUT OF POTASSIUM FROM THE HEART.

BY W. H. HOWELL AND W. W. DUKE.

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IN a previous paper one of us has advanced the hypothesis that the vagus fibres of the heart "end in what may be designated as an inhibitory substance which, under the influence of the vagus impulses, is dissociated with the liberation of potassium compounds to which the phenomenon of inhibition is directly due."¹ To test this hypothesis, namely, that the phenomenon of vagus inhibition is at bottom a case of potassium arrest, the present authors have attempted to determine directly the influence of repeated stimulation of the vagus upon the potassium output in the mammalian heart. For this purpose the heart was isolated, in dogs, rabbits and cats, and kept beating upon a large supply of Locke's solution, fed to the heart through the coronary arteries under oxygen pressure. From time to time the circulation of the stock liquid was suspended and the heart was inhibited for one-half minute to a minute by stimulation of the vagus in the neck, or, as was frequently necessary, by stimulation of a branch from the level of the inferior cervical ganglion on the right side. At the end of the stimulation the heart was washed out through its coronary arteries by a special supply of the circulating liquid, amounting to 75 to 100 c.c. The vagus fibres were stimulated as often as they continued to give a visible inhibition of the auricles, that is, in the different experiments, from five to twenty times, and after each inhibition the heart was irrigated by the same special supply of circulating liquid. At the end of the experiment samples were taken of the circulating liquid which had passed once through the unstimulated heart, and of the special supply which had been circulated repeatedly through the heart after inhibition, and these samples were analyzed for their content in potassium. Control

¹ HOWELL, W. H. : This journal, 1906, xv, p. 280. See also MARTIN: This journal, 1904, xi, p. 370, and BOTAZZI: *Archives de physiologie*, 1896, p. 882.

experiments were made in which precisely the same procedure was followed, except that the heart was not stimulated, or was stimulated through the accelerator nerves, and samples for analysis were taken of the stock liquid which had passed once through the heart, and of the special supply which had been irrigated eight or more times through the heart. In determining the potassium in the samples taken for analysis the method proposed by Cameron and Failyer² was employed. The method consists in destroying the organic matter and driving off the ammonia by incineration, the conversion of the potassium and sodium salts into the corresponding chlorplatinate, the removal of the sodium chlorplatinate by washing with alcohol, and finally the solution of the remaining potassium chlorplatinate in hot water. From the latter salt a double iodide of platinum and potassium is formed by the addition of potassium iodide in acid solution. The double iodide gives a richly colored red-purple solution in which the amount of the potassium contained as chlorplatinate may be accurately determined by comparison in a colorimeter with standard solutions formed from known amounts of potassium chlorplatinate. The method is convenient and yields reliable quantitative results provided the following precautions are observed. 1. The process of incineration should be conducted in platinum crucibles or dishes. 2. In the conversion of the incinerated residue to chlorplatinate the addition of a large excess of platinum chloride must be avoided, and care must be taken in evaporating to dryness to use a low temperature. 3. In washing with alcohol, paper or asbestos filters should not be used; the washing must be conducted by centrifugalization and decantation.

Two series of experiments were made. In the first series of eight experiments the quantity of potassium was not determined by comparison with standard solutions, but the relative amounts of potassium were estimated in the colorimeter by comparison of the solution which had passed once through the unstimulated heart with the solution which had been irrigated repeatedly through the inhibited heart. In every case it was found that the latter solution contained a larger amount of potassium. In five control experiments in which the inhibitory nerves were not stimulated a similar increase was not obtained. The solutions prepared from the two

² See especially SCHREINER and FAILYER, Bulletin 31, U. S. Department of Agriculture, 1906.

samples were either identical in color, or varied so slightly in one direction or the other, that the variation fell within the possible limits of error of the method. In a second series of seven experiments quantitative estimations of the amount of potassium were made by comparison with standard solutions obtained from a carefully prepared specimen of potassium chlorplatinate.³ The results of these analyses are given in the following table. In most cases

Date.	Number of stimulations.	Quantity of liquid used.	Amount of potassium in mgrm. for each c.c.		Increase in potassium in each c.c. of the circulated liquid after vagus inhibition.	Estimated total increase in potassium (?) ²
			Without stimulation.	With stimulation.		
June 3	11 ¹ (?)	c.c. 70	0.155	0.180	0.025 or 16 per cent	mgrm. 1.75
June 7	7	90	0.133	0.156	0.023 or 17 " "	2.07
Oct. 1	7	75	0.139	0.166	0.027 or 19 " "	2.03
Oct. 6	7	45	0.128	0.160	0.032 or 25 " "	1.44
Oct. 12	5	...	0.225	0.250	0.025 or 11 " "	...
Oct. 19	6	60	0.197	0.242	0.045 or 23 " "	2.70
Oct. 26	8	80	0.129	0.167	0.038 or 29 " "	3.04

¹ The heart was stimulated eleven times through the vagus, but it is probable that some of the later stimulations were ineffective.

² The estimate of the total output of potassium can only be considered as approximate. In most of the experiments there was a certain amount of leakage, so that it was impossible to collect the entire amount irrigated through the heart. Moreover it was impossible to prevent a certain amount of dilution of the special supply, since after each irrigation a small amount was left in the heart to be washed away when the stock solution was turned on, and vice versa. Undoubtedly in every experiment only a portion of the increased output of potassium was recovered.

the analyses were made in duplicate with closely concordant results. In contrast with these figures are the results obtained from three control experiments in which the heart was irrigated repeatedly with the same small supply of liquid, but without stimulation of the vagus. The procedure was exactly the same as in the experiments with vagus stimulation. One of these controls was carried

³ This specimen was kindly furnished by Prof. J. H. KASTLE of the U. S. Public Health and Marine Hospital Service, Washington, D. C.

out after a vagus experiment had been performed (June 7), and one before a vagus experiment (October 26).

CONTROL EXPERIMENTS

Date.	Number of circulations.	Quantity of liquid circulated.	Amount of potassium in milligrams.		Increase of potassium in each c.c. of the circulating liquid.
			After one circulation.	After eight circulations.	
June 7	8	c.c. . . .	0.166	0.170	0.004 or 2.4 per cent
Oct. 1	8	75	0.143	0.143	
Oct. 26	8	80	0.129	0.137	0.008 or 6.2 per cent

In the experiment of June 7, the previous vagus stimulation had given an increase of 0.023 mgm. per c.c. or 17 per cent, while in the experiment of October 26, the subsequent vagus stimulation gave an increase of 0.038 mgm. per c.c. or 29 per cent. These results corroborate, therefore, the previous series of five controls in which the accelerator nerves alone were stimulated and in which there was no distinct variation in the potassium contents of the circulated liquid.

It seems evident from these experiments that stimulation of the vagus nerve in the mammalian heart causes a liberation of potassium from the heart substance in a diffusible form. Our experiments have indicated, moreover, that this output of potassium occurs in the auricular end of the heart. This conclusion was forced on us first by the fact that in the rabbit's heart the vagus nerve, in the isolated heart, retains its inhibitory action much longer than in the dog's heart, so that as many as twenty successive inhibitions of the whole heart may be obtained for the rabbit while seven or eight only can be obtained for the dog, and of these only two or three affect the ventricle as well as the auricle. Nevertheless in the single experiment made upon the rabbit, the increase in potassium was very small, a fact which was attributed to the small mass of auricular tissue in this animal compared with the large volume of circulated liquid. In the dog, moreover, only the first two or three stimulations caused stoppage of the ventricle as well as the auricle, the succeeding stimuli, applied as a rule to a branch passing from

the inferior cervical ganglion, to the heart, on the right side, inhibited only the auricles. In these cases, however, the increase in potassium was well marked. The failure of the vagus impulses in these latter cases to stop the ventricle also is to be attributed to the ready assumption of an independent rhythm by the ventricle, when the heart beat is maintained by irrigation with a solution of inorganic salts. We have had frequent opportunities of noticing this fact. Our experience is in accord with the general belief that the vagus impulses affect directly only or mainly the auricular end of the heart. When we remember that the auricular tissue weighs but a few grams, it is apparent that a liberation of as much as 0.4 to 0.5 mgm. of K at each stimulus, within the substance of the auricular tissue itself, is quite sufficient to bring on a condition of potassium inhibition. For the reasons stated in the footnote to Table I, it is more than probable that our method did not enable us to recover all the potassium liberated by the vagus stimulation, and our figures are therefore minimal rather than maximal estimates of the amount of potassium set free in diffusible form at each stimulation. Experiments on the isolated heart show that when the content of the circulating liquid in potassium approaches 0.05 per cent, or even less, the heart ceases to beat, and our experiments indicate that such a concentration of potassium may occur in the auricular tissue, or in certain parts of it, during vagus stimulation. In the paper previously referred to and in a subsequent paper by the present authors,⁴ it was shown that the inhibitory action of the vagus nerve on the heart is markedly favored by an increase in the potassium contents of the circulating liquid, while on the other hand it is markedly diminished by the circulation of a liquid containing no potassium, or indeed may fail altogether under this last condition. Taking these facts in connection with the result obtained in this paper, the authors feel that the theoretical view here advanced, namely, that the vagus stops the heart by causing the liberation of diffusible potassium from some indiffusible compound normally present in the heart tissue, has a convincing amount of evidence in its favor. The view commonly taught of the nature of vagus inhibition, namely, that it is due to the setting up of synthetic or assimilatory processes within the heart substance, while it is conceivable, has little or nothing in the nature of positive evidence to support it. The energy transmitted

⁴ HOWELL and DUKE, *Journal of physiology*, 1906, xxxv, 131.

as a nerve impulse cannot, so far as our actual knowledge goes, be utilized to perform the chemical work of synthesis. This function is fulfilled most probably by the heat energy liberated by the oxidative changes in the tissue, and such changes are characteristic of the nerve impulses which excite functional catabolism rather than of the inhibitory impulses. We conceive that the inhibitory impulses, like the motor or excitatory impulses, act as a liberating stimulus which sets up changes of dissociation, but this dissociation is of such a character that diffusible potassium is set free and through its action the functional catabolism is inhibited.

The authors' conclusion that the increase in potassium in the circulating liquid observed after stimulation of the vagus is due to an increased output of potassium from the heart may be criticised upon several grounds. So far as we can see the following possible objections might be made. The increase may have been due to evaporation occurring in the repeated transfer of the liquid from one receiver to another; it may have resulted from diffusion with the liquids of the heart tissue possibly richer in diffusible potassium than the circulating liquid, or it may have occurred because the action of the vagus fibres caused an absorption of water.⁵ Each of these explanations on examination is found to be insufficient to account for the results obtained. To determine the effect of evaporation alone several determinations of the specific gravity of the liquid used were made at the beginning and the end of an experiment. The determinations were made with a pyknometer and gave such results as the following. Experiment of June 7:

Weight of flask filled with stock liquid	26.6877 gm.
Weight of flask alone	7.8366 "
Weight of liquid	18.8511 "
Weight of flask filled with liquid after eight circulations	26.6900 "
Weight of flask	7.8366 "
Weight of liquid	18.8534 "

The gain in weight of this volume of liquid, as a result of eight circulations through the heart was therefore 2.3 mgm. Since the capacity of the pyknometer was equal to 18.7 c.c., the total increase for the 90 c.c. used in this experiment was equal to

⁵ This last suggestion was made to the authors by Professor MENDEL.

$90/18.7 \times 2.3$ or 11 mgm. If we assume that this increase was due solely to evaporation, the proportional increase in the potassium would be equal to 0.138 mgm., since in the mixture used the potassium chloride constituted 2.3 per cent of the total solid constituents. As a matter of fact the total increase in potassium found in this experiment was equal at least to 2.07 mgm. Similar results were obtained in the other experiments in which the pyknometer was used. With regard to the second objection, namely, that the circulating liquid was enriched with potassium by diffusion from the heart tissue, it is to be remembered in the first place that the heart was always perfused with a litre or more of the circulating liquid before the experiment upon stimulation of the vagus nerve was begun. It is probable, therefore, that by the time that the special supply was turned into the heart, an equilibrium had been established between the circulating liquid used and the heart liquids, in regard to the easily diffusible constituents. Moreover, in the control experiments in which the heart was perfused a number of times with a small supply of the circulating liquid without stimulation of the vagus nerve, no such increase in potassium was observed. In some of these experiments the quantity of potassium was unchanged; in some it showed a slight decrease, and in others a slight increase, the maximal result of this latter kind being an increase of 6.2 per cent, whereas in the vagus experiment on the same heart the increase was equal, at the least, to 29 per cent. With regard to the third objection, namely, that the vagus stimulation may cause an absorption of water and thereby increase the percentage of potassium, the pyknometer determinations, referred to above, are perhaps sufficient to show that such an explanation of the results obtained is entirely inadequate. The circulating liquid, in the experiment of June 7, for example, contained 1.07 gm. of material to each 100 c.c. The potassium in this liquid was increased by 17 per cent as the result of vagus stimulation. If we assume that this increase was due to an absorption of water which should affect alike the concentration of all the substances in solution, then the total increase for these solids would have been from 1.07 to 1.252 gm., that is, an increase of 182 mgm. to each 100 c.c. or 1.82 mgm. to each c.c. According to the pyknometer determinations, however, the total increase in concentration for each c.c. was equal only to 0.123 mgm. Moreover quantitative estimations of some of the other constituents in the solution showed

conclusively, that there was no such increase in concentration, as must have followed, if there had been sufficient absorption of water to raise the concentration of potassium to 17 per cent or more. As stated below, the dextrose showed always a small decrease in concentration, and the calcium, so far as could be determined by the method used, was not changed.

The effect of vagus inhibition on the calcium contents of the circulating liquid. — It was our original intention in planning this series of experiments to determine both the potassium and the calcium contents of the circulating liquid after stimulation of the vagus and of the accelerator. The method used for determining the calcium, which is described briefly below, did not, however, prove to be sufficiently sensitive for our purpose. The minimal variation which could be detected with certainty amounted to 15 to 20 per cent. Within this limit no change in the calcium could be detected as a result of vagus inhibition, but the authors propose to continue this part of the work both for the accelerator and vagus nerves, making use of a more delicate method for the determination of the calcium.

METHODS.

The operation for isolating the heart. — The anæsthetized animal was tracheotomized and connected with an ether bottle. The operation was then performed in the following steps: the upper portion of the sternum and the adjacent first ribs were resected and the internal mammary arteries were ligated; the ribs on both sides were cut away so as to open freely the thoracic cavity; the brachio-cephalic artery was exposed and a cannula was inserted into it to be used subsequently for the inflow of the circulating liquid; the left subclavian artery was ligated at its origin from the aorta, and a ligature ready for tying was laid round the aorta above the origin of the first intercostal arteries; the aorta was cut below this last ligature and while the animal was bleeding the supply of circulating (Locke's) liquid was turned on under moderate pressure through the cannula placed in the brachio-cephalic artery; after sufficient time had been allowed for the washing out of the blood in the heart and aorta, the ligature round the aorta was tied, and thenceforward the supply of circulating liquid passed through the coronary arteries alone; the inferior vena cava was then opened widely to give an outlet to the coronary circulation and the azygos vein and the superior cava were ligated. To prevent an outflow from the pulmonary artery into the lungs, stout ligatures were tied round the roots of the lungs close to the heart. Finally the pericardium was opened along the ventral surface of the heart, and for convenience' sake the permanent outflow cannula was inserted into the superior vena cava, the inferior cava

being ligated close to the heart. The outflow cannula was thrust down far enough to lie within the right ventricle, and it was provided with lateral openings at the level of the right auricle. After the completion of these operations the vagus, or the accelerator nerve, was prepared for stimulation, while the heart was beating upon the artificial circulation. This method of isolating the heart succeeds in practically all cases with dogs, cats or rabbits, the only animals used in these experiments. With the circulating liquid employed, the isolated heart beats very well indeed for a number of hours, provided the aortic valves prove competent for the pressure used, and protect the left auricle and ventricle from over-distension.

The circulating liquid.—As in our former experiments the circulating liquid was made up approximately according to the following formula.

NaCl	0.9	per cent
KCl	0.025	" "
CaCl ₂	0.023	" "
NaHCO ₃	0.02	" "
Dextrose	0.10	" "

In some cases the amount of calcium chloride was increased, and in some of the experiments the amount of potassium chloride was varied. The circulating liquid was saturated with oxygen and was driven into the heart under oxygen pressure. The pressure under which the liquid entered the heart was determined by a mercury manometer attached to the inflow tube. Experience showed that the heart beat best when not fed under too high a pressure. Instead of imitating the normal aortic pressure of 100 to 150 mm. mercury, the circulation was usually maintained under a pressure of from 40 to 60 mm. of mercury. An adequate supply of oxygen in the circulating liquid seems to be quite essential for a successful experiment with the mammalian heart.

Conduct of an experiment.—The circulating liquid was contained in two reservoirs both of which were kept immersed in a large tank of water maintained at a constant temperature of 37° C. One reservoir was large, holding about eight litres, and served as the stock solution on which the heart was kept beating, except during the short periods in which the nerves were being stimulated. The other reservoir was small with a maximum capacity of 250 c.c. The liquid in this reservoir, usually from 75 to 100 c.c., was run through the heart after each stimulation of the nerve. The method of procedure was as follows. When the nerve (vagus or accelerator) was to be stimulated, the supply of circulating liquid was shut off, and the heart was allowed to drain itself as far as possible. The beat of the heart was not interfered with by this temporary cessation of the circulation. After draining for a few seconds the nerve was stimulated, usually for a minute, and then the circulating liquid from the small reservoir was run through the heart, and the outflow was caught in a

special receiver. After the heart had been washed out by this supply, it was again allowed to drain for a few moments, and then the stock supply was turned on and continued until the next stimulation of the nerve. In this way the special supply of liquid in the small receiver was run through the heart a number of times, five to twenty. At the end of the experiment samples of this supply, and of the stock liquid which had been passed once through the heart were taken for analysis. It may be stated that the circulating liquid remained water clear, no matter how often it had been passed through the heart, provided the latter had been thoroughly washed out in the beginning of the experiment. It contained, however, a minute amount of protein material, as shown by the fact that a slight opalescence developed on boiling. It was noteworthy also that liquid which had passed through the heart one or more times developed bacteria readily upon standing in a warm room, whereas the original liquid remained clear.

Stimulation of the nerve. — The vagus nerve was stimulated at first in the neck. In all cases the effect for the first two or three stimulations was a distinct inhibition of the heart, the inhibition in fact was complete except in the case of the cat. With rabbits this portion of the vagus continued to give complete inhibition of the heart for a couple of hours, during which it was stimulated as many as twenty times. In the dog, however, the cervical portion of the vagus lost its effect after two or three stimulations. If the electrodes were then applied to a branch springing from the inferior cervical ganglion on the right side, it was usually possible to obtain five or six additional inhibitions which affected the whole heart or the auricles alone. Stimulation of the nerve below the inferior cervical ganglion on the left side was ineffective after the cervical portion of the vagus had ceased to respond.

Methods of analysis. — The solutions circulated through the heart were analyzed for their contents in potassium and calcium, and in some cases also for the dextrose. For the latter substance Pavy's method was used and gave excellent results. As stated above the solution, after it had passed through the heart one or more times, developed bacteria quite rapidly and suffered in consequence a progressive loss of its sugar. In making the determinations for dextrose, it was necessary, therefore, to examine the solutions immediately at the end of the experiment, or if this was not possible to sterilize the samples by boiling in flasks with a plug of cotton. It was found without exception that the liquid circulated through the heart suffered a slight loss of dextrose. In those cases in which a small supply of the circulating liquid (200 c.c.) was passed through the heart a number of times (6 to 9) the diminution in dextrose was equivalent to 0.5 to 0.6 c.c. of the standard solution used, that is, there was a loss of sugar amounting to 20 to 30 mgm. The analyses for sugar were made only in the earlier experiments, and no data were obtained to indicate whether or not the loss of sugar was specifically influenced by stimulation of the inhibitory or the accelerator nerves.

For the determination of the potassium the authors made use of the colorimetric method described by Cameron and Failyer.⁶ The method involves the formation of a chlorplatinate of potassium and the development from this of a double iodide having a red color by the addition of potassium iodide. The method in detail is as follows. Two cubic centimetres of the solution to be examined were placed in a platinum crucible and $\frac{1}{2}$ c.c. of dilute sulphuric acid (1 to 4) was added. The solution was evaporated to dryness over the water bath, heated over a sand bath until the excess of sulphuric acid was driven off, and was then incinerated over a Bunsen burner at a dull red heat. The ash was moistened with a few drops (10) of hydrochloric acid (acid 1 part, water 1 part) and a few drops (4) of a solution of platinum chloride (1.73 gm. platinum chloride to 25 c.c. water). This solution was slowly evaporated nearly to dryness over the water bath, and the evaporation was then carried just to dryness at a lower temperature. The residue was then washed thoroughly with 95 per cent alcohol by decantation and centrifugalization as follows. Four or five c.c. of alcohol were poured on the residue in the crucible, and the material was carefully rubbed with a glass rod and then decanted into a centrifugalizing tube. Part of the insoluble residue passed into the tube, while a part remained in the crucible. This process of washing was repeated five times, and the crucible containing a portion of the residue was dried over the water bath. The centrifugal tube was placed in the centrifuge for a few minutes, the precipitate being so heavy that a few minutes centrifugalizing enabled one to pour off the supernatant alcohol without disturbing the residue. The latter was then washed several times with a few c.c. of alcohol by centrifugalizing and decanting, and the washed residue was dried over the water bath. The residues in the tube and in the crucible were then dissolved in hot water, enough being used to make a solution of 25 to 50 c.c. After cooling the color was developed in this solution by first making acid with hydrochloric acid, and then adding $\frac{1}{2}$ c.c. of a 25 per cent solution of potassium iodide. The red color which resulted increased gradually to a maximum, and it was found safer to allow the solution to stand ten to twelve hours before making a colorimetric determination. In making this determination the red colored solution was diluted to 100 c.c. so that the original solution, so far as its potassium was concerned, was diluted fifty times. The colored solutions thus obtained from specimens of the circulating liquid, with and without stimulation of the nerves, were compared with each other, and with standard colored solutions obtained from known weights of potassium chlorplatinate. In making the colorimetric determinations the colorimeter devised by Schreiner⁷ was used. It was found to give more satisfactory results than the Duboscq colorimeter.

⁶ Journal of the American Chemical Society, xxv, 1063, 1903. See especially SCHREINER and FAILYER, Bulletin 31, U. S. Department of Agriculture, 1906, p. 31.

⁷ Bulletin 31, U. S. Department of Agriculture, 1906.

The method of procedure given above differs from that described by Schreiner and Failyer in two points, both of which, according to our experiments, are of fundamental importance. In the first place the precipitate of chlorplatينات was washed with alcohol by decantation and centrifugalization. We have found that it is impossible in this process to use filter paper or the asbestos filters recommended by these authors, owing to the fact that some of the platinum chloride adheres so firmly to the filters that it is not removed by the alcohol. Analyses of solutions containing a known amount of potassium salt, when the washing with alcohol was effected through filtration, gave always results that were much too high. It does not seem at all probable that this retention of the platinum salt was due to the presence of ammonia in the filters, since the paper filters were carefully washed and the asbestos was heated to redness in a platinum crucible before using. A similar difficulty was encountered in the use of porcelain crucibles. When the process of incineration and formation of the chlorplatينات was carried out in a porcelain crucible, the quantitative result was invariably too high a yield of potassium as determined by the colorimetric method. Experiments indicated that this result was due to the fact that in washing the residue with alcohol, some of the alcoholic solution of the excess of platinum chloride present adhered firmly to the porcelain surface and was not washed out even by a relatively large excess of alcohol. If the glaze of the crucible was imperfect the error due to this fact was much increased, but it might occur also with porcelain crucibles of the best quality in which the glazed surface was intact. With the alterations in the method that we have described, namely, the use of platinum crucibles exclusively, the avoidance of filters in the process of washing with alcohol, and care in not using too high a temperature in the evaporation of the solution of chlorplatينات, the method has given very satisfactory quantitative results when applied to solutions, such as Ringer mixtures, containing known amounts of potassium. The methods used for determining the calcium in the circulating liquid were not so certain and reliable as in the case of the potassium. We employed the turbidity method described by Schreiner and Failyer.⁸ The method consists in precipitating the dilute solutions of calcium as oxalate and then determining the relative amount of the precipitates by comparing the turbidity of the solutions with the aid of a colorimeter. The authors modified the method to the extent of converting it into a colorimetric turbidity comparison. They found it impossible at times to compare satisfactorily the depth of turbidity alone, but by covering the reflector with a red purple paper the two columns of liquid to be compared were given a reddish tint, the depth of color being less in the tube containing the denser precipitate. By this means we obtained much more accurate comparisons, but at times it also proved unsatisfactory owing to some slight difference in tint which made an exact matching of the tubes impossible.

⁸ *Loc. cit.*

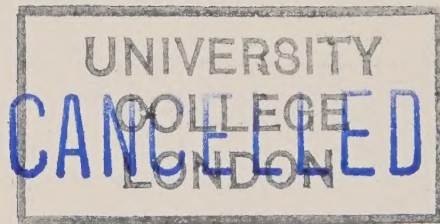
As stated above the results obtained by this method were not satisfactory, and it is proposed to continue this side of the work with the employment of a more sensitive method for determining the calcium.

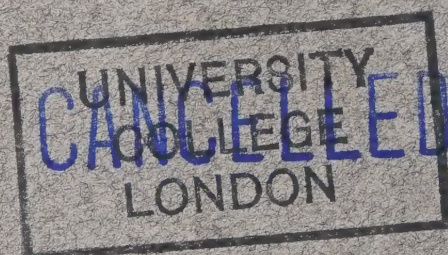
SUMMARY.

1. When an isolated mammalian heart is kept beating upon an artificial circulation of Locke's liquid, stimulation of the vagus nerve causes an increase in the potassium contents of the circulating medium. Under the conditions of the experiment, namely, repeated circulations of a small supply of the liquid after maximal stimulation of the vagus, the increase in the potassium may amount to as much as 29 per cent. It is believed that this increase is referable to an output of potassium from the heart substance, due, probably, to the fact that the inhibitory nerve impulses cause a dissociation of an indiffusible compound in the heart substance and the liberation of the potassium in diffusible form. It is estimated that each stimulus (one half to one minute) may liberate between 0.4 and 0.5 mgm. of potassium. Assuming that the process occurs in the auricular tissue or in a definite portion of the auricles, this amount of potassium should be sufficient to inhibit the heart. The results obtained are presented, therefore, as evidence in favor of the view that the inhibitory action of the vagus nerve upon the heart is mediated through the influence of diffusible potassium compounds set free in the heart by the inhibitory impulses.

2. Stimulation of the vagus nerve causes no detectible change, within the limits of delicacy of the method employed, in the calcium contents of the circulating liquid.

3. Stimulation of the accelerator nerve causes no increase in the potassium contents of the circulating liquid.





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